OUTLINE OF THE PROPOSED WORK FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES

A SYNOPSIS

"Green Synthesis of silver nanoparticles using herbal extracts: Characterization and biological application"

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Abstract:

Nanotechnology has become one of the most promising technologies applied in all areas of science. Nanoparticles (NPs) cause attraction of researchers because of their unique properties, owing to their small size (1–100 nm), large surface-to-volume ratio and increased reactivity. Nanoparticles of gracious metals like platinum, gold and silver and other metals like Zn, Fe, Ti, Pd have received global attention due to their extensive applications in the biomedical and physiochemical fields. Presently, a large number of physical, chemical, and biological methods can be used to synthesize different types of nanoparticles. However, most of the chemical methods are expensive and involve the use of toxic chemicals that pose various biological risks. Therefore, the alternative methods, which are less toxic, eco-friendly and in-expensive are being explored. To meet the increasing demands for commercial nanoparticles new eco-friendly "green" methods of synthesis are being discovered. Plant mediated synthesis of metal nanoparticles is gaining more importance because of its simplicity, rapid rate of synthesis of nanoparticles of attractive and diverse morphologies and Eco friendliness. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles.

It is felt worthwhile to investigate the ability of the plant to synthesize metal nanoparticles and study the properties of the nanoparticles. The synthesized nanoparticles would be characterized by UV-Visible spectrophotometry, Scanning Electron Microscopy, X-ray Diffraction, FTIR Spectroscopy, Transmission Electron Microscopy and Particle size analysis. Synthesized nanoparticles would be subjected to biological Application.

1. Introduction:

The field of nanotechnology is one of the most active areas of research in modern material sciences. Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology. The term "nanoparticles" is used to describe a particle with size in the range of 1nm-100nm[1].

Nowadays, metal nanoparticles have attracted a great scientific interest due to their unique optoelectronic and physicochemical properties with their applications in areas such as molecular diagnostics and drug delivery, imaging, catalysis or sensing[2, 3]. Therefore synthesis of metal nanoparticle through efficient, ecofriendly and cheaper approach is very important in area of research of nanotechnology.

With the growing need to minimize or eliminate the use of environmental-risk substances, as the green chemistry principles describe, the synthesis of nanoparticles using biological entities has received increasing attention in the last decade. The biosynthetic procedures involve either living organisms such as bacteria, fungi and plants[4]. Synthesis of nanoparticles by using this biological entities has been reported successfully but among the use of living organisms plants have found main application as synthesis by using plant is more safe, ecofriendly, cheaper, easy and relatively fast as compared to microbe assisted synthesis[5,6,7,8].

Silver nanoprticles has many important applications. It has wide range of biomedical applications like antibacterial[9], antifungal[10], antimalarial[11], larvicidal[12], anti acne, anti dandruff[13], anti plasmodial[14], anticancer[15], antiwounds activity[16] and in medical devices, including bone cement, surgical instruments, surgical mask[17] and catalytic activity[18]. It is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances. Besides their antimicrobial features, silver nanoparticles exhibit strong optical features making the nanoparticles suitable for biological sensing and imaging. Due to their high conductivity, silver nanoparticles are applied in conductive inks, adhesives and pastes for a range of electronic devices .Silver nanoparticles are also used as catalysts in several chemical reactions such as the oxidation of styrene[19].

The chemical synthesis of silver nanoparticle include chemical compounds like sodium borohydride to reduce Ag+ to AgNPs; however, the chemical methods are often undesirable

because of low biocompatibility due to its associated toxicity. Green synthesis, also called biogenic synthesis, is considered an alternative approach for synthesizing the AgNPs. The process of synthesis starts after incubation of the plant extracts with silver salts (silver nitrate is mostly used). The synthesis of noble AgNPs is a two-step process that first comprises the reduction of Ag+ ions to Ag°, followed by the agglomeration and stabilization that lead to the formation of oligomeric clusters of colloidal AgNPs. The process of reduction takes place in the presence of biological catalysts, Green synthesis of silver nanoparticles via plant extracts[20].

The plant extract act as reducing agent as well as stabilizing agent during the formation of metal nanoparticles due to, the presence of antioxidants (polyphenolic/alcoholic compounds, aldehydes/ketones) and proteins[21].

Large number of active substance present in *Momordica dioica, Pracecitrullus fistulosus, and Gardenia gummifera* played important role as reducing agent and capping agent. Therefore it would be great interest to take advantage of this and explore these different plants for greener synthesis of silver nanoparticle[22,23,24,25].

2. Literature Review:

The extensive literature survey revealed the green method for synthesis of silver and gold nanoparticles

1. **Sivaiah Kummara et al (2016)**
   The present study shows Synthesis, characterization, biocompatible and anticancer activity of green and chemically synthesized silver nanoparticles.

2. **Palanivel Sathishkumar et al (2016)**
   The present study shows Anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesized silver nanoparticles using *Coriandrum sativum* leaf extract

3. **Sujata Patra et al (2015)**
   The present study shows green synthesis of gold and silver nanoparticle and its application in cancer therapeutics.

4. **Partha P. Duttaa et al (2015)**
   The present study shows Antimalarial silver and gold nanoparticles: Green synthesis, characterization and in vitro study.

5. **P.P.N. Vijay Kumar et al (2014)**
   The present study shows Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their anti bacterial activity

6. **K. Narayanan et al (2014)**
   The present study shows Antifungal activity of silver nanoparticles synthesized using Turnip leaf extract (*Brassica rapa* L.) against wood rotting pathogens.

7. **M.Balamurugan et al (2014):**
   The present study shows a novel green synthesis Characterization of Coriander Sativum Mediated Silver Nanoparticles and Evaluation of Its Antimicrobial and Wound Healing Activity.

8. **Mansour Ghaffari-Moghaddam et al ((2014)**
   The present study shows Plant mediated green synthesis and antibacterial activity of silver nanoparticles using *Crataegus douglasii* fruit extract.

3. Aim and Objective:

Aim:

Conventional method for synthesis of metal nanoparticle have many limitation like slower rate of reaction, higher cost and use of chemical reducing agent such as sodium borohydride, dimethyl foramide, trisodium citrate, which can increase burden on environment.

The important consideration in green synthesis are utilization of non-toxic chemicals, use of ecofriendly solvent and use of renewable materials and in this consequences in recent years, green synthesized biocompatible metal nanoparticle are gaining considerable attraction in the field of biomedicine, due to the use of natural resource, rapid rate of synthesis, ecofriendly and safer method.

Other advantage is greener synthesis gives well defined and controlled size of nanoparticle and also it is devoid of contamination and scale up is very easy.

Silver nanoparticle has many application such as antibacterial, antifungal, antimalarial, larvicidal, anti acne, anti dandruff, anti plasmodial, anticancer and antiwounds activity.

In the view of the above, it was felt worthwhile to proceed with **Green Synthesis, Characterization and Biological Activity of silver nanoparticles** which has wide spectrum of biological effects.

Objective:

The objectives of present study are

1) To extract phytoconstituents by using different solvent from selected plant.
2) To develop and establish safe, ecofriendly, cheaper method for the synthesis of silver nanoparticles
3) To characterize synthesized silver nanoparticles with various analytical methods.
4) To evaluate biological activity of the synthesized silver nanoparticles.
5) To study stability of synthesized nanoparticles in different pH range.

4. Methodology to be adopted:

1) Selection of plant and procurement of chemicals

2) Collection of plant

3) Authentication of plant

4) Preparation of plant extract by using different solvent

5) Green Synthesis of silver metal nanoparticle by using plant extract

6) Isolation and characterization of nanoparticle

7) Biological application

8) Stability studies of synthesized nanoparticles

1) Selection of plants: Based on the extensive literature survey and review plant of *Momordica dioica, Pracecitrullus fistulosus and Gardenia gummifera* is planned as green source for synthesis of silver nanoparticles.

2) Collection of plant: The plants will be collected from native place.

3) Authetication of plant: Plant will be then authenticated.

4) Preparation of plant extract: The Dried plant obtained from native will be wash thoroughly with tap water to remove dirt and again wash with distilled water. Plant part will be then ground in the blender and stored for further use. 1g of dried plant part will be boiled in 100 ml solvent for 10 mins and then filter to get extract.

5) Green Synthesis of silver nanoparticle by using plant extract: Aqueous solution (1mM) of silver nitrate will be prepared and used for biosynthesis of silver nanoparticles. 10 mL of plant extract will be added to 95 mL of aqueous solution (1mM) of silver nitrate. It is then boil for 15 mins at 80° C. The formation of silver nanoparticles will then confirmed by UV- visible spectrophotometric analysis. The particles settled down will then thoroughly wash with distilled water for 2 to 3 times to remove the extract from it and then dried in hot air oven. The prepared nanoparticles will then store for further purpose[23].

6) Characterization of metal nanoparticle

a) UV (UV-*vis* spectrophotometry)

b) FTIR (Fourier Transform Infra-red Spectroscopy)

c) XRD (X-ray Diffraction) analysis

d) SEM (Scanning Electron Microscopy) analysis

e) TEM (Transmission Electron Microscopy) analysis

f) DLS (Dynamic Light Scattering) and zeta potential analysis

7) Biological application:

Synthesized nanoparticles will be evaluated for their biological activity like anticancer, antioxidant, catalytic activity

a) Anticancer activity: The *in vivo* and *in vitro* anticancer activity of green synthesized Ag nanoparticles will be evaluated.

b) Antioxidant activity: The antioxidant activity will be checked by DPPH radical scavenging assay.

c) Catalytic Activity: The catalytic ability of green synthesized AgNPs will be investigated using the reduction reaction of Methylene blue by $NaBH_4$ as a model reaction.

8) Stability studies of synthesized nanoparticles:

To check *in vitro* stability of biosynthesized silver nanoparticles, the nanoparticles will be incubated in different physiological buffers and the stability of biosynthesized nanoparticles will be monitored through UV visible spectroscopy[21].

5. Importance of study /society application:

1) The major advantage of green synthesis of nanoparticles is their important role in protecting the environment and also the synthesized particles are stable.

2) This green route of synthesis of nanoparticles is the most convenient, facile, ecofriendly way and minimizes the side effects of chemical and physical methods by avoiding the utilization of toxic chemicals and creating hazardous by-products.

3) The most preferred method for synthesis of nanoparticles is use of biological entities like bacteria, yeast, fungi, and plants; however, plant extracts mediated synthesis is potentially advantageous over microorganisms due to the ease of scale up, safe, simple, and environment friendly. Plant extracts have a broad variety of metabolites that can be used in the reduction of metal ions, capping and stabilizing agent for the nanoparticles synthesis.

4) Metal nanoparticle such as platinum, silver and gold can be used in wide range of biological application such as antibacterial, anticancer, larvicidal, antioxidant etc.

6. Proposed work plan:

Sr. no	Activity	Months
1.	Literature survey	3
2.	Selection, collection and procurement of plant and chemicals	3
3.	Authetification of plant	3
4.	Preparation of plant extract	3
5.	Green synthesis of metal nanoparticles	6
6.	Isolation and characterization of nanoparticles	3
7.	Biological activity of nanoparticles	6
8.	Stability studies of nanoparticles	3
9.	Compilation, interpretation of data & Thesis writing	3
10.	Submission of report	3
	Total	36

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